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PATENT SPECIFICATION

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COMPLETE SPECIFICATION

A Process for Preparing Condiments

We, TAKEDA PHARMACEUTICAL INDUSTRIES LTD., of 27, Doshomachi 2-chome, Higashiku, Osaka, Japan, a body corporate organised under the laws of Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

10 This invention relates to a process for preparing condiments.

It is well known that condiments can be prepared from animal or vegetable proteins by hydrolysing the proteins with acid or protease, and that they contain a variety of amino acids originating from proteins. It is also known that monosodium glutamate and sodium succinate can be used as chemical condiments. In addition to these chemical condiments, sodium 5'-inosinate is also known as an agent to enhance deliciousness or flavour of foodstuffs.

Further, it is known that each of these chemical condiments has its own specificity in taste, and therefore each has gained recognition in its own right. These chemical condiments, when used in combination, enhance the deliciousness or flavour of foods more effectively.

30 Nucleic acid, generally being soluble in water, can be extracted from yeast with an alkaline saline solution. In this case, proteins which are in general hardly soluble in water remain in the filter cake obtained as the result of the extraction.

The yeast extract containing nucleic acid can be hydrolysed into 5'-nucleotides with phosphodiesterase which may be contaminated with adenylic deaminase. Some of 5'-nucleotides are capable of enhancing the deliciousness or flavour of foodstuffs, while 3'-nucleotides which can be produced by chemical hydrolysis of nucleic acid with an acid have no such activity. The hydrolysate of yeast extract is generally contaminated not only with various

impurities which are not toxic or harmful but also with various nucleotides or nucleosides which have no desirable effect. However, it is found that such non-toxic impurities or non-effective nucleosides do not exert an undesirable influence to the deliciousness or flavour of foodstuffs. Therefore the hydrolysate can be used as condiments for foodstuffs without being subject to any refining process.

The filter cake obtained in the extraction of yeast contains, as is mentioned above, proteins which can be hydrolysed with acid or protease into various amino acids. Hence, the hydrolysate of the filter cake naturally contains various amino acids together with non-toxic or harmless impurities. These impurities exert no undesirable influence when the hydrolysate or its processed matter is used as condiments.

Our copending Application No. 10016/59 (Serial No. 912,790) claims a process for hydrolysing ribonucleic acids and/or oligoribonucleotides into one or more 5'-nucleotides, which comprises bringing the starting material into contact at a pH between 4 and 10, with an enzyme system produced by a microorganism capable of producing 5'-phosphodiesterase in the absence of phosphomonoesterase, or in the presence of a phosphomonoesterase inhibitor (which may be the microorganism itself), and separating the 5'-nucleotide or nucleotides produced.

Our copending Application No. 23287/60 (Serial No. 933,826) claims a process for the production of 5'-nucleotides and/or nucleosides, which comprises bringing a ribonucleic acid and/or oligoribonucleotide and/or a 2'-desoxycompound thereof into contact at a pH between 4 and 10 with an enzyme system produced by a microorganism belonging to the order *Sphaeriales*, said enzyme system containing phosphodiesterase, with or without phosphomonoesterase and/or adenylic deaminase.

[Price 4s. 6d.]



subjected





Our copending Application No. 25052/60 (Serial No. 933,827) claims a process for enhancing the flavour of a foodstuff, which comprises adding to the foodstuff at least one of the following, namely 5'-guanylic acid, 5'-uridylic acid, 5'-cytidylic acid or 5'-xanthylic acid or a non-toxic amide or salt thereof.

The present invention provides a process for preparing a condiment for enhancing the taste or flavour of foodstuffs, which comprises extracting yeast with an aqueous solvent, separating the water-insoluble matter from the extract, hydrolysing the yeast extract by means of a phosphodiesterase-containing enzyme system of a microorganism, if necessary removing from the thus obtained hydrolysate any toxic components and/or components having an unpleasant odour or taste, and admixing the resultant composition with a hydrolysate of the water-insoluble matter obtained from the extraction of yeast.

2', 3', and 5'-nucleotides may be formed by the hydrolysis of ribonucleic acid. Among these nucleotides, 5'-inosinic acid has so far been known to be used for enhancing the taste or flavour of foodstuffs. The present inventors have found that 5'-guanylic acid has also a similar effect to 5'-inosinic acid, while most of the other nucleotides have no such an effect. 5'-inosinic acid, however, has not been recognised as an element in the structure nucleic acid, but the deamination product of 5'-adenylic acid. Further, it has been known that the hydrolysis of nucleic acid containing yeast extracts, with acid or other chemical agents, can never give 5'-nucleotides but 2'- or 3'-nucleotides which have no desirable effect. 5'-nucleotides can be produced from yeast extract containing nucleic acids. The processes described in our Applications 10016/59 or 23287/60 (Serial Nos. 912,790 and 933,826) may be used to obtain the nucleotides.

Examples of microorganisms which can be used to hydrolyse the yeast extract are *Streptomyces griseus*, *Streptomyces flavus*, *Streptomyces aureus*, *Streptomyces lavendulae*, *Fusarium roseum*, *Helminthosporium sigmoideum*, *Bacillus brevis*, *Bacillus subtilis*, *Anixiella reticulispota*, *Aspergillus elegans*, *Aspergillus flavipes*, *Botryosphaeria ribis chromogena*, *Chaetomidium japonicum*, *Glomerella cingulata*, *Neurospora crassa*, *Neurospora sitophila*, *Ophiobolus miyabeanus*, *Ophiostoma ulmi*, *Sordaria fimicola* and *Tilachlidium humicola*. In other words, these microorganisms are distributed among the classes *Ascomycetes*, *Fungi imperfecti*, and *Schizomycetes*. Phosphodiesterase is present in all of the enzyme systems produced from these microorganisms, and is capable of hydrolysing nucleic acid into 5'-nucleotides, and some of these enzyme systems contain adenylic deaminase capable of converting 5'-

adenylic acid into 5'-inosinic acid. Examples of microorganisms whose enzyme systems contain adenylic deaminase, are *Streptomyces aureus* and *Streptomyces griseus* and *Aspergillus* microorganisms.

The starting material for preparing the condiments of this invention may be any kind of yeasts such as brewery yeast, bakery yeast, bottom yeast, top yeast, Chinese yeast, wine yeast and food yeast. (Chinese yeast is a yeast which is used in brewing Chinese alcoholic drinks, e.g., *Saccharomyces shaoshung*.)

In the first step, these yeasts are extracted to produce nucleic acid-containing liquor. The extraction is conveniently effected by means of an alkaline saline solution whose pH may be 7.5 to 8.0, and the concentration of common salt may be 0.5 to 5.0%. The extraction is in general promoted by heating at about the boiling point of the saline solution. The mixture can be separated by filtration or by centrifugation into nucleic acid-containing liquor and protein-containing solid matters. The former is used for obtaining a condiment containing 5'-nucleotides, and the latter is used for obtaining an amino acid mixture which is admixed with the above condiment.

In the next step, the nucleic acid-containing liquor is hydrolysed with an enzyme system produced from such microorganisms as afore-mentioned. The enzyme system may be employed for the purpose as a living microorganism, an extracted enzyme solution or a cell suspension. More specifically (i) the microorganism may be incubated on a medium containing yeast extract as above as well as other nutrient sources, or (ii) the filtrate of a culture of the microorganism is brought into contact with the yeast extract, or (iii) a cell suspension of the microorganism is brought into contact with the yeast extract. In all cases, the incubation of the microorganism may be conducted at 20° to 30° C. for 2 to 5 days.

Sometimes the enzyme system is contaminated with phosphomonoesterase capable of hydrolysing 5'-nucleotides into nucleosides which have no taste. In such a case, a phosphomonoesterase-inhibitor is added to the reaction system. The inhibitor may for example be phosphates, metal salts (e.g., salts of nickel or cobalt) or an arsenate or fluoride.

The hydrolysate of the yeast extract is composed mainly of various 5'-nucleotides. Among the 5'-nucleotides, there are contained 5'-guanylic acid and 5'-inosinic acid, both of which are capable of enhancing the deliciousness or flavour of foodstuffs. In cases where the hydrolysate contains no unpleasant taste, odour or toxic or harmful component, it can be used as condiment without being processed any further.

The hydrolysate, however, may contain some proteins or inorganic substances, may be contaminated with substances having unpleas-

Saccharomyces



Aspergillus

ant taste or may contain an odour, any of which make the hydrolysate unsuitable for use for a condiment as such. In such a case, these undesirable matters can be removed by

5 means of suitable ion exchange resin or resins. The resin may be weakly acid cation exchange resin such as carboxylic acid resins, acrylic type resins (e.g., Amberlite IRC-50, XE-89) and phenolic type resins (e.g., Duolite
10 CS-100, available from Chemical Process, Co., U.S.A.) or weakly alkaline anion exchange resin such as polystyrene type, polyamine resins (e.g., Amberlite IR-45) and phenol-formaldehyde type, polyamine resin (e.g.,
15 Amberlite IR-4B). The words "Amberlite" and "Duolite" are Registered Trade Marks. The treatment of the hydrolysate with such an ion exchange resin may be carried out by bringing the hydrolysate into contact
20 with the resin. For the purpose, the hydrolysate is passed through a column or layer of the resin, or is mixed with the resin then filtered. As the components having the ability to enhance the deliciousness or flavour of food-
25 stuffs are hardly adsorbed on the resin while the undesirable impurities are easily adsorbed thereon, the effluent or filtrate is composed of 5'-nucleotides and is almost purified from the impurities which cause undesirable effects.

30 The ability of enhancing the flavour of foodstuffs is synergistically enhanced by the addition of a hydrolysate of the water-insoluble matter obtained as the result of the extraction of yeasts, which has been obtained in the foregoing initial step. The water-insoluble
35 matter is composed mainly of proteins, and therefore is able to be hydrolysed into a mixture of amino acids. The hydrolysis can be effected by *per se* known process. Namely,
40 the water-insoluble matter may be boiled with mineral acid, treated with protease, or heated in an autoclave. As the mineral acid there may be used hydrochloric acid or sulphuric acid, and as the protease there may be
45 used any of the protease originating from microorganisms or animal tissues. The hydrolysate may be admixed as it is with the condiments which have been prepared by the hydrolysis of yeast extracts. On the other
50 hand, it may be treated with an ion-exchange resin to remove the impurities which exert undesirable effects on the taste of foods. The treatment may be carried out in a similar manner to that of the hydrolysate of yeast
55 extract.

The hydrolysate of the water-insoluble matter is then concentrated or dried to obtain the concentrated or dried matter composed chiefly of various amino acids. The amino
60 acids-containing matter is admixed with the condiments obtained by the hydrolysis of the yeast extracts *supra* to give a condiment having an excellent ability of enhancing the deliciousness or flavour of foodstuffs.

65 Of course, both components of the condi-

ment (hydrolysate of the yeast extracts and of the water-insoluble matter) may be mixed with each other before the concentration or drying of these components or after these treat-
70 ments. In general, the mixing is carried out before the treatment so as to easily give a homogeneous mixture.

The condiments may be added to enhance the deliciousness or flavour to soups, fermented foods, pasty foods, sauces, salads,
75 vinegared foods, canned foods or pickled vegetables, in the course of their cooking or manufacture or just before eating.

The following examples further illustrate the present invention:—
80

EXAMPLE 1

Ten kilograms of dried brewery yeast is extracted under heating at 90° C. for 4 hours with 150 litres of a 3% saline solution the
85 pH of which was previously made 8.0 with sodium hydroxide. The extract is separated centrifugally from insoluble matters.

A strain of *Streptomyces griseus* (Krainky emend. Waksman et al.) Waksman et al. (this strain has been deposited at Institute for
90 Fermentation, Osaka, Japan and American Type Culture Collection, Washington, D.C., U.S.A. under the respective accession numbers of IFO-3430 and ATCC-10137) is incubated for 24 hours at a temperature of
95 28° C. on an aqueous medium, whose pH is 7.0, composed of 5.0% of soluble starch, 3.0% of cornsteep liquor, 0.1% of ammonium sulphate, 0.05% of hydrous magnesium sulphate, 0.5% of peptone and 0.2% of calcium carbonate.
100

To 150 litres of the yeast extract prepared as above there is added 50 litres of the culture filtrate of the above culture. The hydrolysis is then carried out at a tempera-
105 ture of about 40° C. for 15 hours by keeping the mixture standing. The hydrolysate is filtered after admixing 500 grams of diatomaceous earth (Hyflo super cell, the word "Hyflo" being a Registered Trade Mark),
110 and then is passed through a column packed with 10 litres of a cation exchange resin to obtain 220 litres of effluent. The effluent is neutralised, is concentrated under reduced pressure, and then is spray dried to obtain
115 550 grams of powdery condiment which contains 5'-guanylic acid, 5'-inosinic acid and other 5'-nucleotides.

In this example, strongly acid polystyrol sulphonic acid type bead resin sold under
120 the trade name of "Amberlite IR-120" from Rohm & Haas, Co., U.S.A. was used as the cation exchange resin, the word "Amberlite" being a Registered Trade Mark.

The insoluble matter obtained above by
125 centrifuging the yeast extract 1 is boiled for 20 hours together with 20 litres of a diluted hydrochloric acid (20%), then is filtered. After being neutralised with diluted sodium

hydroxide, the filtrate is admixed with the effluent from the ion exchange resin. The mixture is concentrated under reduced pressure, then is dried by spraying to obtain 700 grams of a powdery condiment which contains various amino acids together with 5'-nucleotides.

EXAMPLE 2

Instead of the microorganism used in Example 1, any of the following microorganisms may be employed. A similar result to that of Example 1 is obtained in each of the cases when any of the following microorganisms is employed.

- 15 * *Streptomyces albogriseolus* Benedict, Shotwell et Pridham NRRL B-1305
- * *Streptomyces aureus* (Waksman et Curtis) Waksman et Henrici IFO-3303, ATCC-13404
- 20 *Streptomyces viridochromogenes* (Krainisky) Waksman et Henrici Waksman's strain
- * *Streptomyces purpureus* Lindenbeim IFO-3389, NRRL B-1454
- 25 * *Streptomyces coelicolor* (Müller) Waksman et Henrici IFO-3807, ATCC-13405
- * *Helminthosporium sigmoideum* var. *irregulare* Cralley et Tullis IFO-5273, ATCC-13406
- 30 * *Bacillus brevis* Migula emend. Ford ATCC-8185
- Bacillus subtilis* Cohn emend. Prazmowski IFO-3032, ATCC-13407
- 35 *Anixiella reticulispota* Saito et Minoura IFO-5483, ATCC-13828
- * *Aspergillus elegans* Gasperini IFO-4286, ATCC-13829
- Aspergillus flavipes* (Bainier et Sartory) Thom et Church IFO-4052, ATCC-13830
- 40 *Aspergillus fischeri* Nehmer IFO-5866, ATCC-13831
- Aspergillus melleus* Yukama IFO-4339, ATCC-13832
- 45 *Aspergillus nidurans* (Eidam) Winter IFO-5713, ATCC-13833
- * *Botryosphaeria ribis chromogena* G. et D. IFO-4837
- 50 * *Chaetomidium japonicum* Saito et Okazaki IFO-4451, ATCC-13835
- * *Glomerella cingulata* (Stromm.) Spauld, et v. Schr. IFO-5907, ATCC-13836
- * *Neurospora crassa* Shear et Dodge IFO-6067, ATCC-13837
- 55 *Neurospora sitophila* Shear et Dodge IFO-6069, ATCC-13838
- * *Ophiobolus miyabeanus* Ito et Kuribayashi IFO-4870, ATCC-13839
- * *Ophiostoma ulmi* (Buisman) Nannf. IFO-6128, ATCC-13840
- 60 * *Sordaria fimicola* (Rab.) Cesari et de Notaris IFO-4846, ATCC-13841

* *Tilachidium humicola* Oudemans IFO-5696, ATCC-13842.

In the examples presented above, numbers 65 attached to the names of species of microorganisms show the respective accession numbers of the strain used in Institute for Fermentation, Osaka, Japan (IFO), Northern Utilisation Research Branch of U.S. Department of Agriculture, Peoria, Ill., U.S.A. (NRRL) or American Type Culture Collection, Washington, D.C., U.S.A. (ATCC).

In the list of microorganisms in this example, microorganisms marked with asterisk (*) 75 produce phosphodiesterase accompanying phosphomonoesterase therewith. Therefore, when these microorganisms are employed, phosphomono-esterase-inhibitor is preferably 80 added when the yeast extract is hydrolysed. When sodium arsenate is used as the phosphomono-esterase-inhibitor, the concentration of the inhibitor in the reaction mixture may be about 6×10^{-3} moles per litre.

WHAT WE CLAIM IS:—

1. A process for preparing a condiment for enhancing the taste or flavour of foodstuffs, which comprises extracting yeast with an aqueous solvent separating water-insoluble matter from the extract, hydrolysing the yeast extract by means of a phosphodiesterase-containing enzyme system of a microorganism, if necessary removing from the thus obtained hydrolysate any toxic components and/or components having an unpleasant odour or taste, 95 and admixing the resultant composition with a hydrolysate of the water-insoluble matter obtained from the extraction of yeast.

2. A process as claimed in Claim 1, wherein the microorganism belongs to the class 100 *Ascomycetes*, *Fungi Imperfecti* or *Schizomycetes*.

3. A process as claimed in Claim 1 or Claim 2, wherein the removal of the toxic components and/or components having an unpleasant odour or taste is effected by means 105 of one or more ion-exchange resins.

4. A process as claimed in any of Claims 1 to 3, wherein the yeast is extracted with a saline solution of pH 7.5 to 8.0. 110

5. A process as claimed in Claim 4, wherein the saline solution is heated.

6. A process as claimed in any of Claims 1 to 5, wherein a phosphomonoesterase inhibitor is added to the hydrolysis system. 115

7. A process as claimed in any of Claims 1 to 6, substantially as described with reference to either of the examples.

8. Condiments when produced by a process as claimed in any of the preceding claims. 120

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ATCC

Patent

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4. A process as claimed in any of Claims 1 to 3, wherein the yeast is extracted with a saline solution of pH 7.5 to 8.0.

5. A process as claimed in Claim 4, wherein the saline solution is heated.

6. A process as claimed in any of Claims 1 to 5, wherein a phosphomonoesterase inhibitor is added to the hydrolysis system.

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